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We have identified a gene on chromosome 16 which restores senescence to human and rat mammary tumor cells by functional complementation. Initial efforts at mapping, using PCR based analysis of rat revertant clones arising from senescent colonies, revealed that the senescence gene was located within a 3-7 cM consensus deletion region at 16q24.3. A yeast artificial chromosome (YAC) contig of this consensus deletion region was obtained and analyzed for marker content. Two overlapping YACs, 792_E_1 and 624_H_7, spanning this region were retrofitted with a selectable marker. Transfer of these two YACs by spheroplast fusion restored senescence to immortal rat ovarian tumor cells. Indirect transfer of retrofitted YAC 792_E_1 by microcell mediated chromosome transfer restored senescence to immortal human and rat mammary tumor cells. These results indicate that SEN16 is located on a 360 kb YAC which maps to 16q24.3. Experiments to further map and clone this gene are in progress.

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PI - Signature Date

Deepthi E. Reddy
187-74-0476

TABLE OF CONTENTS

ANNUAL REPORT

INTRODUCTION

Normal mammalian cells exhibit a limited proliferative potential (1). At the end of their replicative span, these cells senesce, becoming enlarged, vacuolated and flattened. These cells are metabolically active but are in a state of irreversible G1 arrest and do not divide (2-4). Cellular senescence is a genetically programmed event which is controlled by genes whose collective effect manifests at the end of the cell's life span. In contrast to normal cells, breast cancer and other tumor cells can multiply indefinitely, having escaped senescence as a result of alterations of critical genes (5,6,7). Escape from senescence thus represents an important step in tumor progression. Since senescence genes are involved in negative regulation of cell growth, they are regarded as a class of tumor suppressor genes. The main focus of this project is identification, localization and high resolution mapping of the gene(s) involved in the restoration of senescence to immortal breast tumor cells.

Cytogenetic and loss of heterozygosity (LOH) studies on breast tumors suggest the presence of tumor suppressor genes on human chromosomes 1, 3, 6, 11, 13, 16, 17, 19 and 20 (8,9,10). Increased frequency of LOH and chromosomal deletions on the long arm of human chromosome 16 at 16q22, 16q24 and 16q24-qter has been reported in breast cancer, prostate cancer and hepatocellular carcinoma (11-21). These data suggest an important role for a gene(s) on chromosome 16 during the conversion of normal breast epithelium into a cancerous state.

To determine the effect of the introduction of normal human chromosome 16 into breast cancer cells, intact normal human chromosome 16 was introduced into human and rat mammary carcinoma cells by means of microcell mediated chromosome transfer method (MMCT). Human breast cancer cell lines SKBR-3 and MCF7 and rat mammary tumor cell lines NMU and LA7 were used in these experiments. Chromosome transfer clones showed morphological and growth characteristics typical to senescent cells. Continuous cultivation of the senescent cells in selection media gave rise to revertant clones which are morphologically similar to and have the same growth rate as the immortal parent cells. These revertant clones were shown to have lost the region of the introduced human DNA that was capable of restoring senescence to the tumor cells. PCR analysis of 5 human revertants for the presence of previously mapped polymorphic chromosome 16 specific markers localized the senescence gene to the long arm of chromosome 16. Transfer of human chromosome 13 into MCF7 and NMU and transfer of human chromosome 7 into LA7 did not restore senescence to these cells.

A sub-monochromosomal hybrid library developed in Dr. Athwal's lab consists of mouse A9 cells that contain fragments of human chromosome 16 tagged with the *gpt* selectable marker. Nine hybrid clones containing fragments of human chromosome 16 were analyzed cytogenetically and by PCR using previously mapped chromosome 16 markers to determine the size and position of the fragment on chromosome 16. Two fragments of chromosome 16 containing regions 16q22-qter and 16q23-qter were selected for further chromosome transfer studies.

Introduction of these fragments of chromosome 16, containing either the region 16q22-qter or 16q23-qter also induced senescence in both the human and rat breast tumor cells.The same phenotype was observed in several independent experiments. Furthermore, these fragments induced senescence in ovarian cancer cells and in SV40 transformed human and mouse fibroblast cells. These results confirm the involvement of 16q22-qter in restoration of senescence to immortal tumor cells by functional complementation.

Initial analysis of five rat (mammary tumor) revertant clones with fifteen chromosome 16 specific markers had localized the senescence gene to 16q24.2-16q24.3. Further analysis with 30 rat revertants using an additional 60 markers from 16q23-qter was done. A consensus deletion map was derived from the presence or the absence of these markers in the revertant clones. This

consensus deletion region contains 21 markers. Based on information from genetic and physical maps of these markers available from various databases, we mapped the region of the senescence gene (SEN 16) to a 3-7 cM region at 16q24.3.

The remaining fragment of chromosome 16q23-qter present in a rat revertant clone of LA7 which lost the region of the chromosome that harbored the senescence gene, was transferred into the human breast tumor cell line, MCF7. This fragment of human DNA did not restore senescence to the human breast tumor cells. These results indicate that the same region of chromosome 16, perhaps the same gene at 16q24.3, restores senescence in both rat and human immortal tumor cells.

BODY

EXPERIMENTAL METHODS

Cell Lines and Growth Conditions

The cell lines used are human breast tumor cells, MCF7, SKBR-3 and MDA-MB 468 (ATCC); rat mammary tumor cell line LA7 (22); mouse mammary tumor cell line JC (ATCC); rat ovarian cancer cell line, ROV12 (ATCC); and, mouse immortal epithelial cell line, A9 (ATCC). All cultured were maintained in DF12 medium supplemented with 10-15% FBS at 37^{0}C in 7.5% CO_2 incubators. YAC transfer clones are grown in 400 µg/ml of G418 containing media.

Yeast Media

All YAC cultures were grown in synthetic dextrose (SD) media supplemented with 14g/liter casamino acids. Retrofitted YACs were grown in CM media supplemented with 20 mg/l uracil, 40 mg/l tryptophan but does not contain adenine.

Retrofitting of YAC Clones

The retrofitting method used by us is essentially as described in (23), using a plasmid pRAN4. This plasmid carries a *neo* selectable marker as well as a yeast ADE gene and is designed to integrate by homologous recombination into the URA gene in the right arm of the pYAC4 cloning vector, converting YAC clones from Ura+ Ade- Trp+ to Ura- Ade+ Trp+. After screening for the desired Ura- phenotype, all retrofitted clones were examined by pulsed field gel electrophoresis (PFGE) to confirm retention of the YAC insert and then transferred onto filter paper for southern blotting to demonstrate colocalization of the *neo* marker with the human insert (Fig.2).

Spheroplast Fusions

Spheroplasts were prepared as described (24) with some modifications. A 50 ml culture of the desired YAC clone was grown to 2X10^7 cells per ml in synthetic medium containing uracil and tryptophan but without adenine. Pelleted yeast cells were resuspended in SCEM buffer (10 mM sodium citrate/1 mm EDTA/1M sorbitol/30 mM ß-mercaptothanol) containing 45 µl of 10 mg/ml Zymolyase 20-T(ICN biologicals) and incubated at 30^{0}C. Spheroplast formation was monitored by optical density or observing under microscope. Spheroplast pellet was resuspended in 2 ml of STC(1M sorbitol/ 10mM $CaCl_2$/10mM Tris pH 8.0). 2 x 10^6 cultured recipient cells were trypsinized, washed and mixed with 10^8 spheroplasts in 0.1 ml of medium. 0.5 ml of PEG-1500 (50%, Boehringer Mannheim) containing DMSO was added and the mixture incubated at 37^{0}C for

90-120 sec. The fusion reaction was diluted with 5 ml of serum free DMEM, incubated at room temperature for 10 min, centrifuged at 100g for 5 min and plated in 4-5 100 mm^2 plates in DMEM containing 10% serum. After 24 hours medium was replaced with DMEM+ 10% FBS and 400ug/ml of G418. G418 resistant clones were selected , isolated, propagated and observed for morphological changes.

Lipid Transfection (Lipofection)

All lipofections were carried out using the Lipotaxi mammalian transfection kit from Stratagene per manufacturer recommendations. Briefly, 10 µg of YAC DNA was incubated in an activated solution containing 2.9 ml of serum free media and LipoTAXI transfection reagent for 30-45 min. This mixture was diluted to 8 ml and added dropwise onto a layer of 60-70% confluent recipient cells. After, 4-6 hours of incubation at 37^{0}C and 7.5% CO_2, 2.5 ml of media containing twice the normal serum concentration was added to the plate and incubated overnight. The next day, the medium was replaced with fresh, complete medium and incubated for 24 hrs. Cells were split in a ratio of 1:2 the next day and 400 µg/ml of G418 was added to the cells for selection.

Analysis of YAC Transfer Clones

All transfer clones were analyzed for morphological and growth characteristics typical to senescence i.e., enlarged, flattened and vacuolated cells. PCR analysis was done to determine the presence or absence of the introduced*neo* gene and markers known to be present on the YAC.

Senescence Associated β–Galactosidase Staining

For β-galactosidase detection (29), senescent cells are washed with PBS and fixed in 2-3% formaldehyde for 3-5 mi. At room temperature. The fixed cells are washed with PBS again and incubated at 37^{0}C in 3 ml of freshly prepared staining solution which contains 1 mg/ml X-gal, 40 mM citric acid/sodium phosphate buffer, pH 6.0: 5 mM potassium ferrocyanide; 5 mM potassium ferricyanide; 150 mM NaCl and 2 mM magnesium chloride. The stained slides are examined under bright field low power microscope. Positive staining is seen in 12-16 hours.

RESULTS AND DISCUSSION

Isolation of YAC Clones

In order to further localize SEN 16, seven yeast artificial chromosomes (YAC) shown to span the consensus deletion region were obtained from Research Genetics (Huntsville) for further studies. The size of YACs 624_H_7, 706_H_5, 707_F_9, 720_E_2, 792_E_1 and 897_G_6, and information on their chimerism is given in Table 1. Unfortunately, database searches did not provide additional information about the presence of chromosome 16 markers on these YACs. Therefore, PCR analysis was performed (with the chromosome 16 specific primers that were used earlier to analyze revertants) on all of these YACs to determine the markers that each YAC carried (Fig. 1). Three chimeric YACs, 624_H_7, 792_E_1 and 897_G_6, contained 13 markers out of the 21 present in the consensus deletion at 16q24.3. The other 8 markers from the consensus deletion did not map to any of the YACs analyzed. The remaining four YACs contained markers that were located outside the consensus deletion region (Fig. 3).

Retrofitting of YAC Clones

Mammalian genes present on YAC clones can be expressed when introduced into cultured mammalian cells (25-28). Since replicative senescence is a dominant phenotype over immortal

growth, a YAC containing the senescence gene, when introduced into tumor cells should restore senescence to these cells. For selection of the introduced YAC after transfer into the mammalian cells, the YACs were first retrofitted with a selectable marker, *neo*. All retrofitted clones were examined by pulsed field gel electrophoresis (PFGE) to confirm retention of the YAC insert and then blotted to demonstrate colocalization of the *neo* marker with the human insert (Fig. 2).We were able to retrofit all YACs (Table 2) except 897_G_6. YAC 792_E_1 fragmented during retrofitting to yield two derivative clones, 792E1 t2 and 792E1 t3 (Fig. 2). STS marker analysis was done on these derivative clones to determine the region of chromosome 16 present on them (Fig. 3).

Direct Transfer of Retrofitted YACs into Mammalian Cells

The retrofitted YAC clones, 624H7t8, 792E1 t2, 792e1t3 and 706H5t5 were transferred into several cell lines (MCF7, SKBR-3, MDA-MB468, LA7, JC, A9 and ROV12) by spheroplast fusion and lipofection. Unfortunately, mammalian cells are refractory to the transfer of the YACs as seen in our laboratory and as reported by others. No colonies were recovered from any of the human tumor cell lines analyzed.

Lipofection transfer of 792E1t3 and 624H7t8 into ROV12 and LA7 gave rise to several independent colonies that grew in G418 containing media. However, PCR analysis on these transfer clones showed that these clones did not contain the *neo* gene or the YAC DNA. The same result was obtained in several experiments with both cell lines. The exact mechanism by which the colonies obtained in this series of experiments demonstrated G418 resistance is unclear. Therefore, this method of transfer was not used for further experiments.

YAC transfers by spheroplast fusion were partially successful but the efficiency of transfer into mammalian cells proved to be highly variable. Successful transfers were obtained only into the cell lines A9, LA7 and ROV12 (Table 2). The number of colonies obtained, phenotype and length of survival of the YAC transfer colonies is shown in Table 3 and PCR analysis of all YAC transfer clones is described in Tables 4 and 5.

Spheroplast Transfer of 624H7t8

This YAC, when transferred into ROV12 restored senescence to these cells. Transfer clones showed characteristic features of senescence. Senescence associated β-galactosidase staining of these cells stained blue indicating enzyme activity typical to senescent cells (Fig. 7). Furthermore, PCR analysis on these clones with chromosome 16 specific markers and primers for the *neo* gene showed the presence of introduced YAC DNA (Table 4). 624_H_7 is a chimeric YAC containing DNA from chromosomes 1q32, 3p21 and 16q24.3 as determined by FISH analysis. Although the senescent clones contain a portion of chromosome 16 as seen by PCR, the possibility that the chromosome 1 and chromosome 3 portions are responsible for induction of senescence cannot be excluded.

Transfer of this YAC into LA7 gave 4 colonies in one experiment. This experiment could not be reproduced despite several attempts. The phenotype of the transfer clones was similar to that of the parental cells. However, PCR analysis (Table 4) showed that these clones did not retain any of the introduced YAC DNA and contained only the *neo* gene. This experiment was thus inconclusive.

Transfer of this YAC into A9 failed to yield any colonies despite several attempts even though this cell line gave G418 positive clones with the other YAC transfer experiments (24-27; unpublished results from our lab).

Spheroplast Transfer of 792E1t2 and 792E1t3

YACs 792E1t2 and 792E1t3, when transferred into ROV12 restored senescence to these cells. Five colonies were obtained in the transfer of 792E1t2 into ROV12 while 792E1t3 gave 24

colonies (Table 3). Transfer clones showed characteristic features of flattened, enlarged and vacuolated cells. Senescence associated β-galactosidase staining of these cells stained blue typical to senescent cells. Furthermore, PCR analysis (Table 5) of these clones with chromosome 16 specific markers and primers for the *neo* gene confirmed the presence of introduced YAC DNA (Table 4). 792_E_1 is a chimeric YAC containing DNA from chromosomes 1, 11 and 16. The chromosomal location of the region from chromosomes 1 and 11 is not known while STS marker analysis showed that it contained DNA from 16q24.3. The retrofitted derivatives contain truncated chromosome 16 DNA compared to the original YAC and have lost the DNA from chromosome 1. However, the role of chromosome 11 causing senescence in these cells cannot be ruled out.

Transfer of both of these derivative clones into LA7 failed to produce any clones despite repeated attempts.

Transfer of 792E1t3 into A9 did not give any positive colonies while the transfer of 792E1t2 gave 8 colonies. This YAC did not alter the growth characteristics of the parental cells and PCR analysis showed the presence of the introduced DNA. One of these clones, 792t2(A9)4-1 which contains intact introduced YAC, was subsequently used for indirect transfer of the YAC DNA into breast tumor cells.

Spheroplast Transfer of 706H5t5

Transfer of YAC 706H5t5 into LA7 gave only a single G418 positive colony and transfer into A9 gave 4 such colonies. This YAC did not affect the indefinite proliferation or morphological phenotype of either cell line.

Indirect Transfer of Retrofitted YAC Clones into Tumor Cells

Since direct transfer of the YACs into human and rat mammary tumor cells was fraught with technical problems, indirect transfer of the YACs by MMCT was attempted as an alternative approach. A schematic of the strategy used is shown in Fig. 4. The 2 A9 clones 792t2(A9)4-1 (containing 792E1t2) and 966A9L1 (containing a region of chromosome 6) were used in these experiments.

When the 792t2(A9)4-1 clone was microcell transferred into LA7, a total of 60 colonies were recovered in four experiments (Table 5). All colonies displayed the characteristic senescent phenotype identical to that previously observed following the introduction of chromosome 16 into these cells. Cells showed flat, enlarged cell morphology, diminished growth rate and progression to complete growth arrest (Fig. 6). A total of 25 colonies, which were obtained in MCF7 cells following the transfer of the same YAC (Table 5), showed the senescent phenotype (Fig. 6). In addition to morphological changes, these colonies were analyzed for senescence associated (SA)-β -gal activity at pH 6.0. All senescent colonies showed β-gal activity at pH 6, while parental tumor cells (Fig.7) and immortal clones did not. PCR analysis of transfer clones showed the presence of the introduced YAC (Table 7).

When 966A9L1 was used as microcell donor to transfer chromosome 6 sequences into LA7 cells, all eight colonies recovered in one experiment showed immortal cell growth without any changes in cell morphology (Table 6). These results provide a good negative control for the experiments to transfer 792t2(A9)4-1 into the tumor cells. No colonies were obtained with this YAC in MCF7 cells.

In addition to a portion of chromosome 16, the YAC 792t2 contains chromosome 11 DNA. However, chromosome 11 does not induce senescence in breast cancer cells (30,31). So the role of this chromosome in the restoration of senescence to these cells can be ruled out. Therefore, we conclude that the YAC 792t2 (360 kB) carries a senescence gene from chromosome 16 that restores senescence to immortal human and rat mammary tumor cells.

CONCLUSIONS

Mammalian cells are refractory to direct YAC transfer as reported by others and in our experience. We have overcome these difficulties by designing a method of indirect transfer of the YAC DNA to target cells. Using this method we have identified a 360 kb YAC at 16q24.3 which restores senescence to immortal human and rat mammary tumor cells. Further mapping and cloning of this gene will aid in understanding the mechanisms of cellular senescence and tumor progression.

FUTURE GOALS AND EXPERIMENTS

Further mapping of the senescence gene at 16q24.3: We have purchased a PCR-based Bacterial Artificial Chromosome (BAC) library (Research Genetics). BAC clones are smaller in size, easy to handle and show only 1-2% chimerism. This library will be screened for BACs that contain the markers present on 792e1t2 and the consensus deletion region in order to create a contig of this region. In addition, the BACs will be retrofitted with a selectable marker for transfer into breast tumor cells to identify complementing BAC clone. These results will provide a defined position of the senescence gene and aid in the final cloning of the gene.

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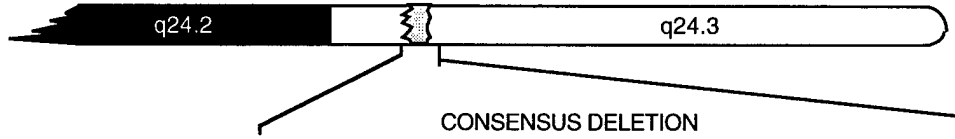
Fig. 1. PCR analysis of YACs with chromosome 16 specific markers



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897_G_6	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
707_F_9	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
720_E_2	—	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
688_F_11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—
706_H_5	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

+ Positive for marker
— Negative for marker

Table 1: Size and Chimerism information of YACs obtained

YAC	Size	Chimerism
624_H_7	400 Kb	Chr. 1, 3 and 16 (FISH)
688_F_11	630 Kb	unknown
706_H_5	420 Kb	unknown
707_F_9	1770 Kb	unknown
720_E_2	450 Kb	unknown
792_E_1	360 Kb	Chr. 1, 11 and 16 (database)
897_G_6	1420 Kb	Chr. 1, 3, 5, 8 and 16 (database)

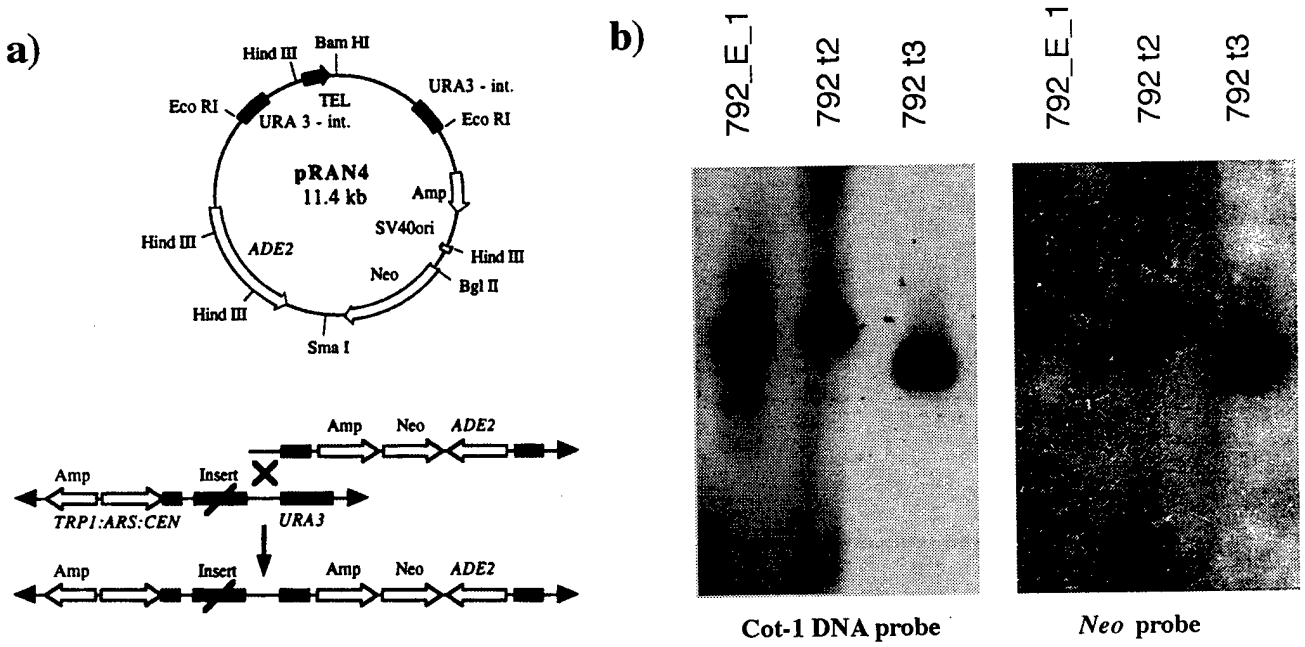


Fig.2a. A schematic representation of homologous recombination of pRAN4 into a YAC constructed with pYAC4. The pRAN4 segment replaces a part of the right arm of YAC vector following homologous recombination (Markie et. al., 1993; Somat. Cell. Genet. 19: 161-169).
Fig.2b. Southern blot hybridization of parental and two retrofitted YAC clones of 792e1 following hybridization with human Cot-1 DNA. The same blot was rehybridized with neo-probe to show hybridization specific to retrofitted clones only.

Fig. 3: Partial YAC Contig of the Consensus Deletion Region Harboring SEN 16

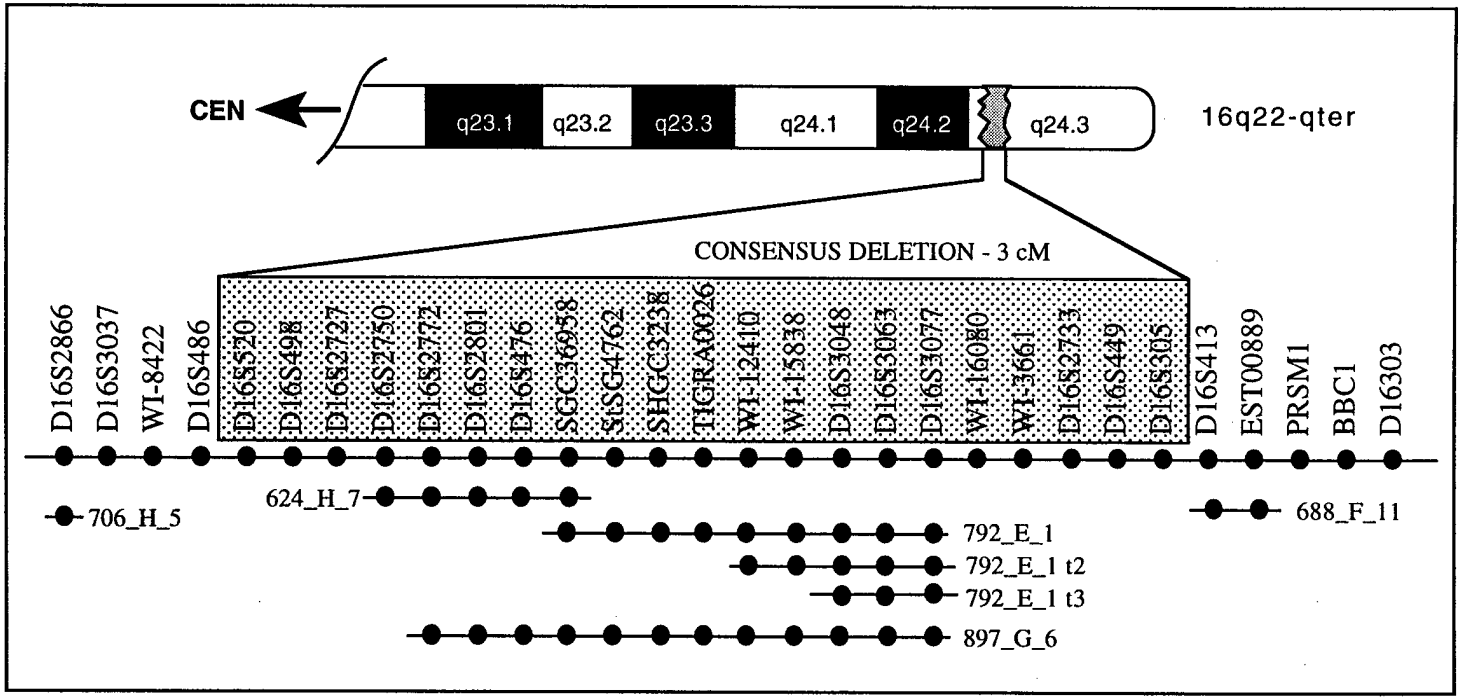


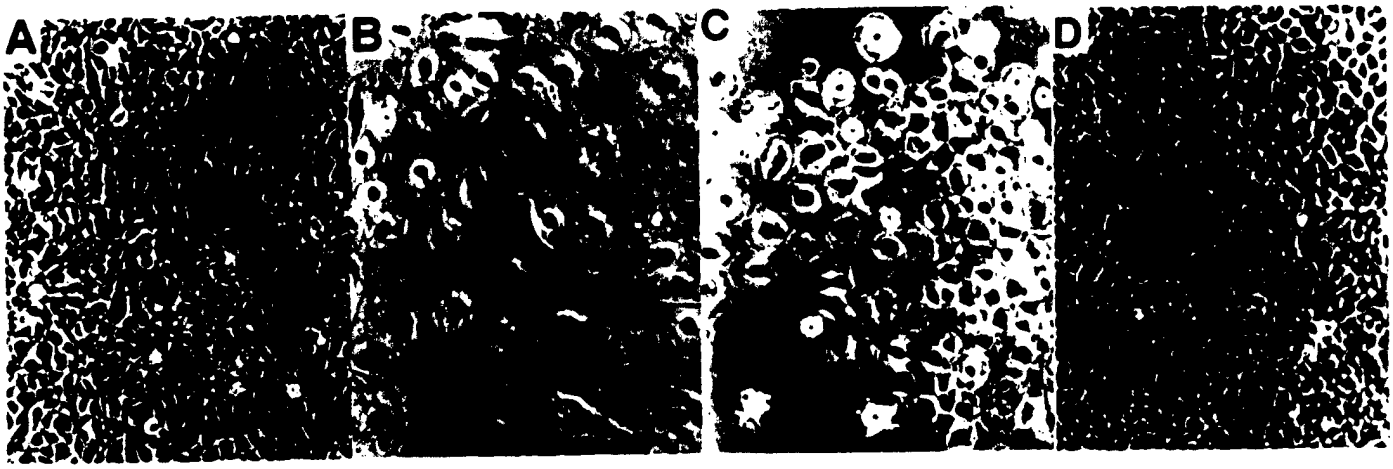
Table 2: Successful spheroplast fusions of YACs with respective cell lines

YAC	Successful Fusion	No Fusion
624H7 t8	ROV12	A9 and LA7
792E1 t2	ROV12 and A9	LA7
792E1 t3	ROV12	A9 and LA7
706H5 t5	A9 and LA7	ROV12

Table 3: Number of colonies obtained and phenotype of YAC transfer colonies

YAC	Cell Line	Total Colonies	Phenotype Sen./Imm
624H7 t8	ROV12	19	Senescent
	LA7	4	Immortal
792E1 t2	ROV12	5	Senescent
	A9	8	Immortal
792E1 t3	ROV12	24	Senescent
706H5 t5	LA7	1	Immortal
	A9	4	Immortal

Fig. 5 Transfer of YACs into ROV12 by Spheroplast Fusion



ROV12 | ROV12 X 624H7 t8 | ROV12 X 792E1 t2 | ROV12 revertant

Table 4: PCR Analysis on 624H7 t8 transfer Clones

MARKER	624_H_7t8	ROV12	ROV(624t8)S1	ROV(624t8)S2	ROV(624t8)S3	ROV(624t8)S1-1sb
NEO	●	○	●	●	●	●
D16S2750	●	○	●	●	●	●
D16S2801	●	○	●	●	●	●
D16S2772	●	○	●	●	●	●
D16S476	●	○	●	●	●	●

624h7 t8 X ROV12

MARKER	624_H_7t8	LA7	LA(624t8)p1-1	LA(624t8)p1-2	LA(624t8)p1-3	LA(624t8)p1-4
NEO	●	○	●	●	●	●
D16S2750	●	○	○	○	○	○
D16S2801	●	○	○	○	○	○
D16S2772	●	○	○	○	○	○
D16S476	●	○	○	○	○	○

624h7 t8 X LA7

●- Positive for marker analyzed
○- Negative for marker analyzed

Table 5: PCR Analysis on 792E1 t2 and 792E1 t3 Spheroplast Transfer Clones

MARKER	792_E_1t2	ROV12	ROV(792t2)#R1	ROV(792t2)#R2	ROV(792t2)#R3	ROV(792t2)mix
NEO	●	○	●	●	●	●
D16S3077	●	○	●	●	●	●
D16S3063	●	○	●	●	●	●
D16S3048	●	○	●	●	●	●
WI-12410	●	○	●	●	●	●
WI-15838	●	○	●	●	●	●

792E1 t2 X ROV12

MARKER	792_E_1 t2	A9	A9(792t2)4-1	A9(792t2)4-2	A9(792t2)4-3	A9(792t2)4-4	A9(792t2)4-5	A9(792t2)4-6	A9(792t2)4-7	A9(792t2)15mix	A9(792t2)15-1
NEO	●	○	●	●	●	●	●	●	●	●	●
D16S3077	●	○	●	●	●	●	●	●	●	○	○
D16S3063	●	○	●	●	●	●	●	●	●	○	○
D16S3048	●	○	●	●	●	●	●	●	●	○	○
WI-12410	●	○	●	●	●	●	●	●	●	○	○
WI-15838	●	○	●	●	●	●	●	●	●	○	○

792E1 t2 X A9

MARKER	792_E_1t3	ROV12	ROV(792t3)D1p2	ROV(792t3)C1p6	ROV(792t3)S1p2	ROV(792t3)S2p1	ROV(792t3)S3p1	ROV(792t3)S4p2	ROV(792t3)R1p2	ROV(792t3)R2p2	ROV(792t3)R3p2	ROV(792t3)C8p22
NEO	●	○	●	●	●	●	●	●	●	●	●	●
D16S3077	●	○	●	●	●	●	●	●	●	●	●	●
D16S3063	●	○	●	●	●	●	●	●	●	●	●	●
D16S3048	●	○	●	●	●	●	●	●	●	●	●	●

792E1 t3 X ROV12

●- Positive for marker analyzed
○- Negative for marker analyzed

Fig. 4: Strategy for indirect Transfer of YACs into Immortal Mammary Tumor Cells by microcell mediated chromosome transfer (MMCT)

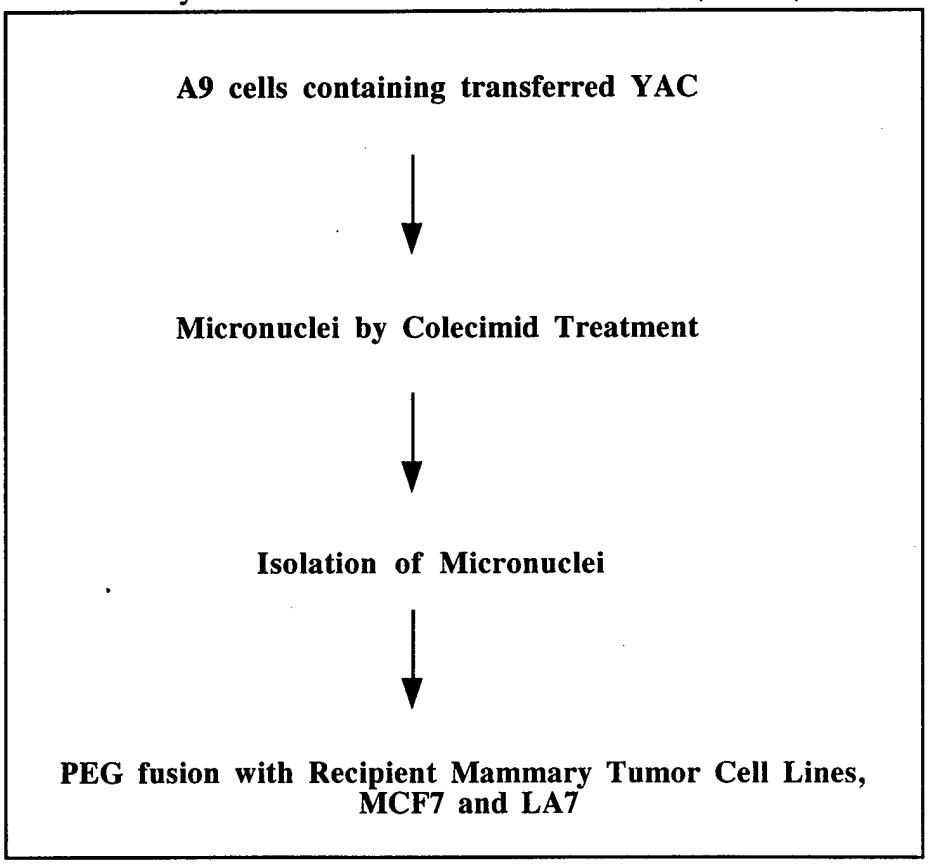
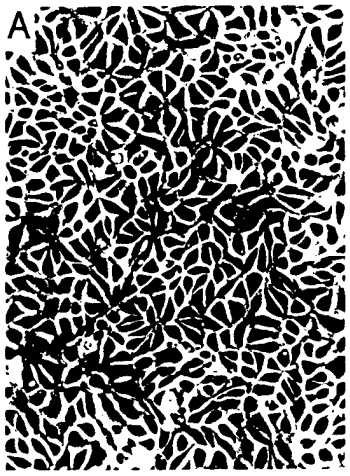
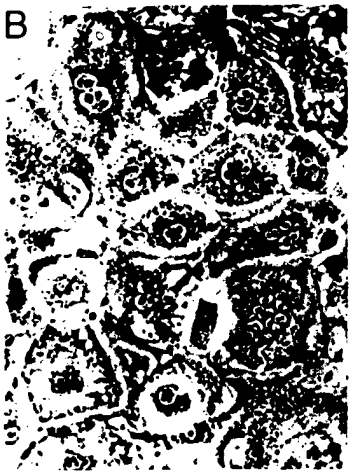


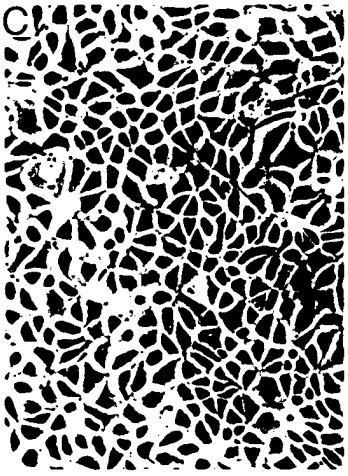
Fig. 6 YAC Transfer into Immortal Breast Tumor Cells



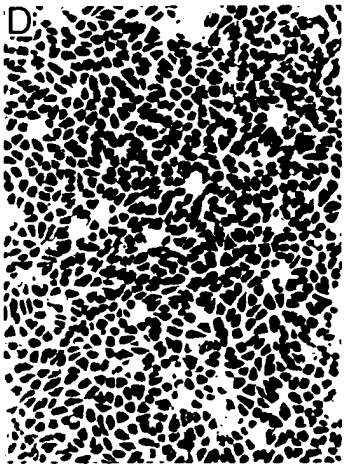
LA7



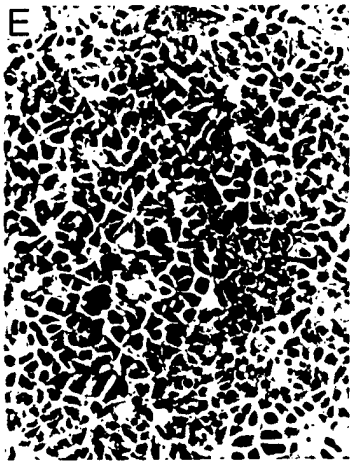
LA7 X 792t2(A9)4-1
sen



LA7 X 792t2(A9)4-1
rev



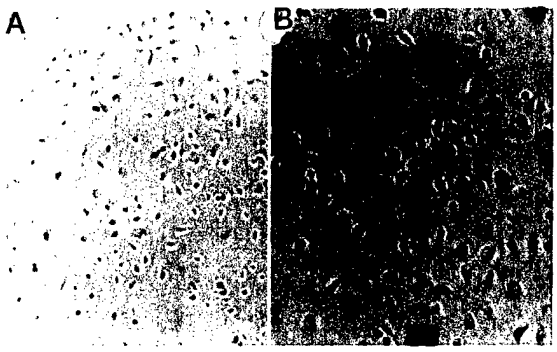
966A9L1



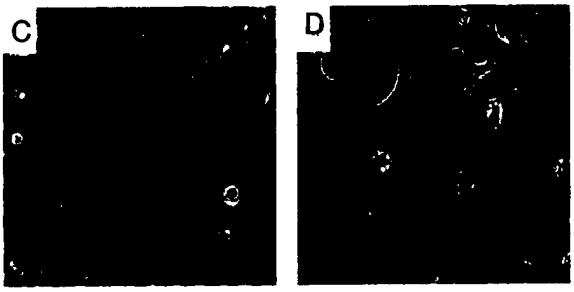
MCF7

MCF7 X 792t2(A9)4-1
sen

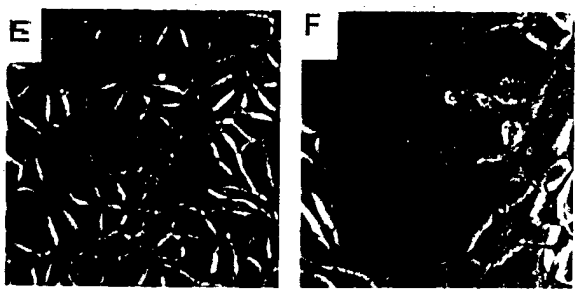
Fig. 7 Senescence Associated β-gal Staining of Senescent YAC Transfer Clones and Parental Tumor Cells



ROV12 ROV12 X 624H7 t8



MCF7 MCF7X 792t2(A9)4-1



LA7 LA7X 792t2(A9)4-1

Table 6: YAC Transfer by MMCT - number of clonoies obtained, phenotype and length of survival of YAC transfer clones

YAC	Cell Line	Num. of Expts.	Total # of Colonies	Phenotype / Survival (days)
792t2 (A9)4-1	LA7	5	60	Senescent (30 - 40)
	MCF7	5	25	Senescent (50 - 80)
966A9L1	LA7	1	8	Immortal
	MCF7	1	0	-

Table 7: PCR analysis of YAC transfer clones

MARKER	792_E_1t2	792t2 (A)4-1	MCF7	MCFX 792t2(A9)4-1sen	LA7X 792t2 (A9)4-1sen	4-1LAF1C1	4-1LAF2S2	4-1LAF2C1	4-1LAF2R3	4-1LAF2R5	4-1LAF3C2	4-1LAF3C3	4-1LAF3C3	4-1LAF4S1	4-1LAF4C1	4-1LAF4C3	4-1LAF4C4	4-1LAF4D1	792t2LA(4-1)A1-1	792t2LA(4-1)A1-2	LA(4-1A)F3p12 mix	LA7
NEO	●	●	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
D16S3077	●	●	●	NP	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
D16S3063	●	●	●	NP	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
D16S3048	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
WI-12410	●	●	●	-	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○
WI-15838	●	●	●	-	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○

NP - not polymorphic
● - Positive for marker analyzed
○-negative for marker analyzed